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EVIDENCE FOR HYBRIDIZATION OF TWO OLD WORLD *RHAMNUS* SPECIES—*R. CATHARTICA* AND *R. UTILIS* (RHAMNACEAE)—IN THE NEW WORLD

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ABSTRACT. A highly variable, fully fertile population of *Rhamnus* that was first established in Ann Arbor, Michigan, circa 1952 was studied to identify the species present and determine whether some individuals were of hybrid origin. Specimens of four taxa hypothesized as being present in the population—*R. cathartica, R. davurica* var. *davurica, R. davurica* var. *nipponica,* and *R. utilis*—were used to establish reference clusters using a canonical discriminant analysis. Results of the discriminant analysis suggest that the population is a hybrid swarm and that only *R. cathartica* and *R. utilis* were involved in the formation of the hybrids at the study site. Additional analyses with the computer program HYWIN corroborated the results of the discriminant analysis, enabled circumscription of putative hybrids, and for each hybrid provided a pair of specimens in the data set that best matches the morphologies of its parents. Utilization of both discriminant and HYWIN analyses in studies of hybrid swarms appears complementary and effective.

Key Words: Rhamnaceae, *Rhamnus, R. utilis, R. cathartica,* introduced species, hybrid swarm, North America, Michigan, discriminant analysis, HYWIN

Rhamnus is a wide-ranging genus comprising up to 150 species of Temperate and Tropical zones of both hemispheres. It is abundant in eastern Asia and southwestern North America and adjacent Mexico (Rehder 1940; Brizicky 1964; Johnston and Johnston 1978; Cronquist 1981; Mabberley 1987). In North America the genus is represented by about 25 species, including several introductions from Europe (Wolf 1938).

A highly variable, fully fertile population of *Rhamnus* was discovered by Reznicek in 1984 in Washtenaw County, Michigan. This population included some plants that clearly were *R. cathartica* L. Yet, some individuals with larger, narrower leaves resembled the Asian species *R. davurica* Pall. var. *davurica* or *R. davurica* var. *nipponica* Makino. Other individuals with larger, longer blades, but short petioles, resembled *R. utilis* Decne. [*R.*

davurica Pall. var. *davurica*—*R. citrifolia* (Weston) W. J. Hess & Stearn var. *citrifolia,* not *R. citrifolia* Rusby (Hess and Stearn 1979, 1981). *Rhamnus davurica* var. *nipponica* Makino—*R. nipponica* (Makino) Grubov (1949a); *R. citrifolia* (Weston) W. J. Hess & Stearn var. *nipponica* (Makino) W. J. Hess & Stearn (1979); *R. davurica* subsp. *nipponica* (Makino) Kartesz & Gandhi (1994)]. A substantial number of plants could not be placed reasonably with any of these species and were suspected of being hybrids. All four taxa are closely related members of subsection *Cervispina* Moench (Weberbauer 1895; Wolf 1938; Grubov 1949a), and all have the chromosome number $2n = 24$, as determined by Dolcher (1963), Mulligan (1961), and Wulff (1939) for *R. cathartica*; Li (1988) for *R. davurica* var. *davurica* and *R. davurica* var. *nipponica*; and Bowden (1945) for *R. utilis.*

Rhamnus cathartica is native in Europe and western and northern Asia east to northwestern China. Though rarely cultivated now, it was introduced to North America presumably as an ornamental woody plant or perhaps for its fruits, which have been considered as having medicinal value. Also, extracts of the drupes at various developmental stages treated with corresponding mordants yield a wide range of colors that can serve as dyes, and the bark is rich in tannins used as tanning agents (Grubov 1949b; Mabberley 1987). *Rhamnus cathartica* has escaped from cultivation, become naturalized in eastern North America, and now is abundant in urban environments and nearby woodlands (Greene 1896; Bailey 1947; Barnes and Wagner 1981). In recent years it has become a serious pest. It is also the alternate host for the oat rust, *Puccinia coronata* Corda (Okane et al. 1990; Swink and Wilhelm 1994). *Rhamnus davurica* var. *davurica* is distributed in eastern Russia, eastern China, and Korea. *Rhamnus davurica* var. *nipponica* is distributed in central and northern Japan (primarily Honshu) as well as eastern China and Korea. *Rhamnus utilis* is distributed in central and eastern China, Korea, and is rare in Kyushu, Japan (Makino 1904; Wolf 1938; Rehder 1940; Steward 1958; Grubov 1949b; Kitagawa 1979; Ohwi 1984; Flora of China Editorial Committee 1996). Grubov (1949a) considered specimens identified as *R. davurica* var. *nipponica* from Korea, western China, and adjacent Russia to represent the segregate *R. ussuriensis* J. J. Vassil., restricting *R. davurica* var. *nipponica* (under *R. nipponica*) to Japan. We have kept them in our analyses under *R. davurica* var. *nipponica. Rhamnus davurica* and *R. utilis* are

economically important in the manufacture of dyes (Mabberley 1987).

No pertinent records documenting the introduction of *Rhamnus* into the study site are available. Herbarium specimens of *R. cathartica* in MICH document its presence in Michigan as early as 1914. A collection from a site near the study area in 1980 (*Reznicek 6165*, MICH) was the basis for the first report of *R. utilis* in Michigan (Voss 1985). *Rhamnus davurica* var. *davurica* and *R. davurica* var. *nipponica* are not known from Michigan. Elsewhere in eastern North America *R. davurica* (as *R. citrifolia*) is reported by Gleason and Cronquist (1991) as "sparingly introduced into our range." Swink and Wilhelm (1994) reported both *R. utilis* and *R. davurica* (including var. *nipponica*) for the Chicago region. *Rhamnus davurica* var. *nipponica* also was reported from Massachusetts (Seymour 1982).

The objectives of this study were to: (1) identify the species present at the site, especially to ascertain whether any individuals were, in fact, *Rhamnus davurica* var. *davurica, R. davurica* var. *nipponica* or *R. utilis*; (2) assess the nature and extent of hybridization at the study site; and (3) attempt to circumscribe possible hybrids.

MATERIALS AND METHODS

Sampling of the study specimens. Vigorously grown terminal branches, bearing sun-leaves and drupes, were sampled to represent the variation present at the study site. The plants occur along ca. 150 meters on the southeast-facing edges of a hedgerow of *Gleditsia triacanthos* L. and *Maclura pomifera* (Raf.) C. K. Schneid. (see Appendix 1 for locality and specimen citations). Because fruits offer several characters that distinguish species, only trees bearing drupes were sampled. Additional sampling was done ca. 10 meters east of the hedgerow in clusters of trees and shrubs dominated by *Rhamnus* spp. and surrounded by mowed grass. The clusters were established around trees of *Malus pumila* Mill. surviving from a former orchard. Drupes were preserved in FAA. Thirty-two specimens were collected and deposited in MICH. The principal plant associates were: *Lonicera maackii* (Rupr.) Maxim., *Prunus serotina* Ehrh., *Viburnum opulus* L., *Celastrus orbiculatus* Thunb., and *Ligustrum vulgare* L., with *Morus alba* L., *Acer negundo* L., and *Rosa multiflora* Thunb. as occasional

associates. The site is at an elevation of 265–271 meters on a heavy, clay-loam soil derived from glacial till, and is within U.S.D.A. Hardiness Zone 5: minimum temperatures of −29°C to −23°C (U.S.D.A. 1990).

Determination of age. Transverse sections from the nine *Rhamnus* trees having the largest trunks in the sampling area were taken at ca. 30 cm above ground in June 1994, and annual rings were counted. These nine trees were not necessarily the trees sampled for morphology. On the basis of observations on seedling growth in the area, three years were added to the count to cover the period of development to a height of 30 cm. Vouchers and wood samples are deposited in MICH.

Specimens and characters selected. Thirty-eight drupe-bearing herbarium specimens of *Rhamnus cathartica* (MICH), *R. davurica* var. *davurica, R. davurica* var. *nipponica,* and *R. utilis* (A, MICH; Appendix 1) served as reference specimens and were studied with the study specimens.

Seventeen qualitative and quantitative characters were measured on each specimen. The characters selected were those that showed variation among the taxa represented by the reference specimens, including characters used by Schneider (1916), Rehder (1940), and Grubov (1949a). Two to 20 measurements were made for each character, depending on the quality of the specimen. Ten drupes were sampled from each specimen. The mean of each of the quantitative characters was entered into the data set. The characters measured were: (1) maximum length of terminal blade; (2) maximum width of terminal blade; (3) maximum length/maximum width ratio of terminal blade; (4) petiole length of terminal leaf; (5) petiole length/blade length ratio of terminal leaf; (6) sum of the basal angles of terminal blade; (7) gloss of the adaxial surface of terminal blade (scored on a scale of 1–5 where 1 = dull and 5 = very glossy); (8) pubescence on the adaxial surface of terminal blade; (9) pubescence on the abaxial surface of terminal blade (both 8 and 9 scored: 0 = none, 1 = none or along the major veins, 2 = along the major veins, 3 = throughout or only along the major veins, and 4 = throughout); (10) thorn length (apex to the base of the first terminal bud); (11) thorn pubescence (scored: 0 = absent and 1 = present); (12) peduncle length; (13) drupe length; (14) drupe width; (15) drupe

length/width ratio; (16) drupe color (scored on a scale of 1–5 where 1 = green and 5 = purple); (17) the number of pyrenes per drupe (scored: **0** = 2 pyrenes, **1** = 2 or 3, **2** = 3, **3** = 2, 3, or 4, **4** = 3 or 4, and **5** = 4). Blade gloss and drupe color of the study specimens were scored while the material was fresh. Characters 7 and 13–16 were not available on the reference specimens and were entered as missing. Raw data are available from the authors.

Statistical analysis. *A priori* groups corresponding to the taxa (*Rhamnus cathartica, R. davurica* var. *davurica, R. davurica* var. *nipponica,* and *R. utilis*) that might have played a role in the hybridization were established using the reference specimens. These specimens were included in the discriminant analysis, described below, as reference points to allow comparison and positive identification of the study site's putative parental species (Appendix 1). Reference specimens 1–10 were *R. cathartica*; 12, 18, 20–22, 24, and 25 were *R. davurica* var. *davurica*; 11, 14, 19, 23, and 26–30 were *R. davurica* var. *nipponica*; and 15–17 and 31–39 were *R. utilis* (specimen 13 was omitted). An additional specimen (40, MICH) was collected near the study site and, suspected of being a hybrid, was included in the analysis with the study specimens. A stepwise linear discriminant analysis was performed to select the characters that are most predictive for distinguishing these groups. It was followed by a canonical discriminant analysis employing the first two canonical variables, using the MIDAS package on the University of Michigan mainframe computer. The derived discriminant function then was used to assess the positions of the study specimens and specimen 40 with respect to the reference specimens. Results were plotted (Figure 1) using the SAS statistical package version 6.08 (SAS Institute, Inc. 1993) on a DOS personal computer.

Although Atchley et al. (1976) note serious problems with the extensive use of ratios in statistical analyses, three ratios that are diagnostic were used in the discriminant analysis so that both it and the HYWIN analyses (see below) use the same suite of characters. This may cause some distortion of the clusters presented in the discriminant function analysis, but facilitates comparisons among the analyses.

Determination of putative hybrids and parental specimens. The complexity of the data set and the limitations of discriminant functions prompted the development of the computer program HYWIN (Estabrook et al. 1996). This program evaluates triplets of specimens in which one specimen is considered the hybrid offspring of the other two, and ranks the hypothesized hybrid according to character intermediacy, parental distance, and equality. The HYWIN analysis of the data set used the default weights (wI = 1, wE = 1, and wP = 1) for the ranking criterion and the 0.95 probability option. Two major HYWIN analyses were conducted. In the comprehensive analysis, 55 specimens (all 32 study specimens, specimen 40, and 22 reference specimens representing *Rhamnus cathartica* and *R. utilis*) were analyzed. The objective was to generate another set of hypotheses on the identity of the specimens and their grouping without *a priori* designation of putative parental species. These hypotheses then can be compared with those generated by the discriminant analysis. In the second analysis, only the study specimens (41–72) were analyzed. This was followed by a third analysis in which the specimens that were suggested as putative hybrids in the second analysis were removed.

RESULTS

Determination of age. Four of the nine trees whose rings were counted were 35–42 years old and ranged from 44.7 to 81.5 cm in circumference (14.2–25.9 cm in diameter). Those trees were a 42-year-old staminate *Rhamnus cathartica,* a 38-year-old carpellate *R. cathartica,* a 36-year-old staminate *R. cathartica,* and a 35-year-old carpellate *R. utilis*. The remaining five trees appeared to be hybrids, were 27–34 years old, and ranged in circumference from 37.6 to 73.7 cm (12–23.5 cm in diameter).

Statistical analysis. Four clusters corresponding to the reference specimens of the four taxa were generated (Figure 1).

Characters 1, 3, 5, and 6 were found to be best for distinguishing *a priori* groups. Ranges of these characters are listed in Table 1.

Study specimens (marked by black dots in Figure 1) were placed in clusters corresponding to the taxa as follows: in the *Rhamnus cathartica* cluster 41, 44, 53, 55, 61, 67, 68, and 72; in the *R. utilis* cluster 42, 46, and 66; and in the *R. davurica* var.

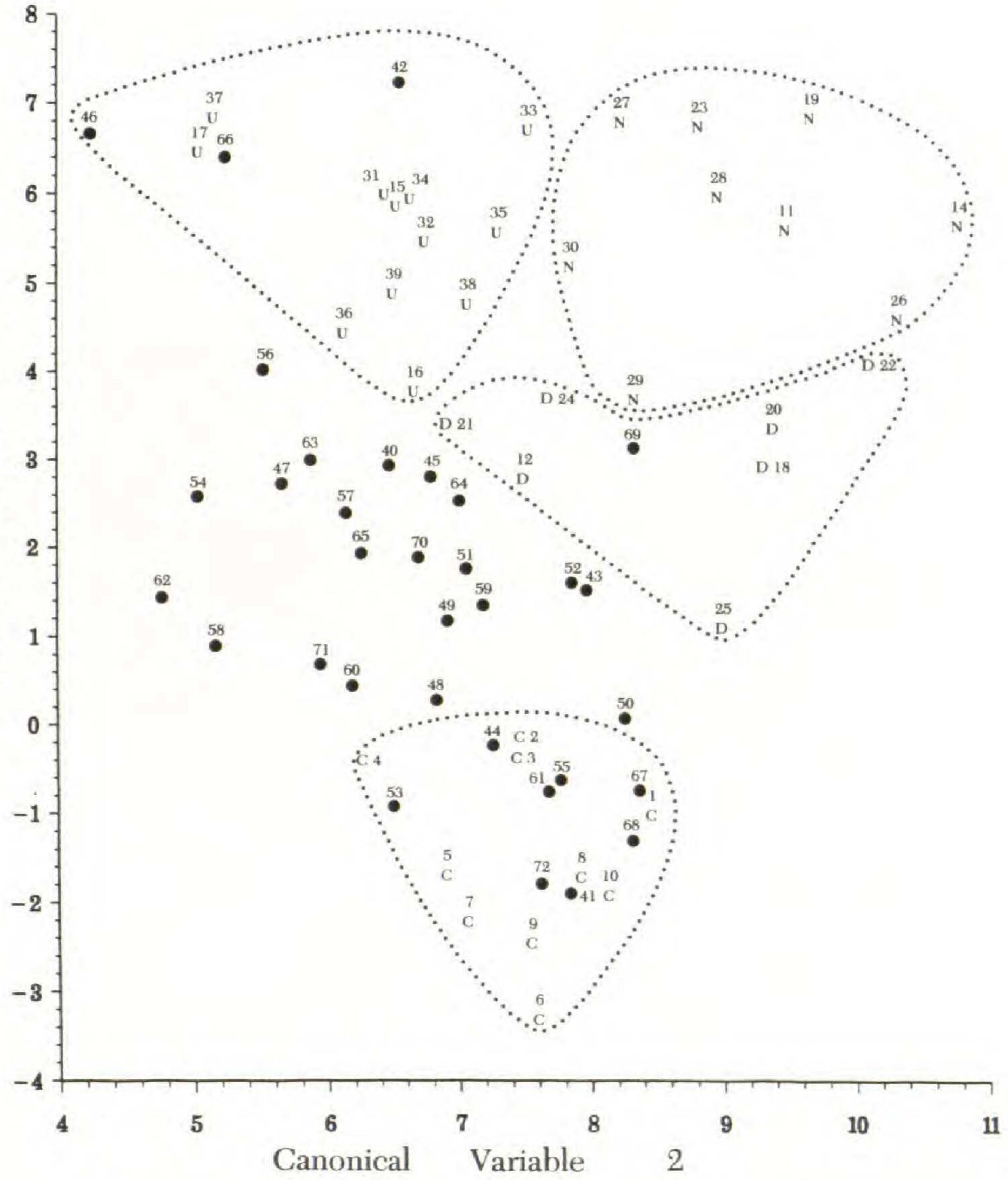

Figure 1. A canonical discriminant analysis of 72 *Rhamnus* specimens employing the first two canonical variables. Letters denote assigned group membership of the reference specimens: C = *R. cathartica*; D = *R. davurica* var. *davurica*; N = *R. davurica* var. *nipponica*; U = *R. utilis*; ● = specimen from the study site (plus specimen number 40). The number above or beside each letter or dot is a case number representing the specimen (see Appendix 1). Dotted outlines of groups are merely an aid to visualizing clusters of taxa and have no statistical significance.

davurica cluster 69. No study specimens were placed in the *R. davurica* var. *nipponica* cluster. Specimen 40 and all other study specimens were arrayed between the clusters formed by specimens referable to *R. cathartica* and *R. utilis*. These included specimens 43, 45, 47–52, 54, 56–60, 62–65, and 70–71.

Table 1. The ranges of characters found best for distinguishing *a priori* groups in the discriminant analysis.

Character	Taxon			
	R. cathartica	*R. utilis*	*R. davurica* var. *davurica*	*R. davurica* var. *nipponica*
1. Maximum length of terminal blade (mm)	19–66	66–159	60–125	68–124
3. Maximum length/maximum width ratio of terminal blade	1.2–2.5	2.2–3.4	2.2–3.5	2.9–4.4
5. Petiole length/blade length ratio of terminal leaf	0.08–0.75	0.03–0.23	0.14–0.58	0.01–0.23
6. Sum of the basal angles of the terminal blade	81°–170°	27°–127°	61°–125°	33°–100°

Comprehensive HYWIN analysis. The count for the 0.95 probability option for the specimens examined was the 374 highest ranking triplets. Twenty-one of the 55 specimens never participated in the role of a putative hybrid (reference specimens 2, 4–10, 15, 17, 31–34, and 37; and study specimens 42, 46, 48, and 66–68), and 34 specimens were suggested as putative hybrids (reference specimens 1, 3, 16, 35, 36, and 38–39; specimen 40; and study specimens 41, 43–45, 47, 49–65, and 69–72). Input and output data are available from the authors.

HYWIN analysis of the study specimens. Results are presented in Table 2 and summarized by frequency counts in Table 3. The count for the 0.95 probability option for the specimens examined was the 201 highest ranking triplets. Twelve of the 32 specimens never participated in the role of a putative hybrid (41, 42, 44, 46, 48, 55, 61, 63, 66–68, and 72), and 20 were suggested as putative hybrids (43, 45, 47, 49–54, 56–60, 62, 64, 65, and 69–71). Two specimens, 46 and 68, were suggested as parents in the highest ranking triplets of ten specimens suggested as hybrids, specimens 66 and 68 were suggested as parents in the highest ranking triplets of five specimens, and other combinations of putative parents were each suggested in the highest ranking triplets of the remaining five specimens. A striking feature of Table 2 is that specimen 68 (*Rhamnus cathartica*) was suggested as one of

the putative parents of 17 of the 20 specimens that were suggested as putative hybrids. The equality score of eight of these specimens indicates that they are closer in their overall morphology to specimen 68. Of these, specimen 60 is the closest to specimen 68 (NP = 0.176). Specimen 46 (*R. utilis*), the second most frequent putative parent, was suggested as one of the putative parents of 11 of the 20 specimens. The equality score of five of these specimens indicates that they are closer in their overall morphology to specimen 46. Of these, specimen 69 is the closest specimen to specimen 46 (NP = 0.277; Table 2).

Comparisons among the analyses. Results of the discriminant analysis and the comprehensive HYWIN analysis on the status of the reference specimens substantially agree (Table 2). However, a few reference specimens were suggested as hybrids in the HYWIN analysis. In the *Rhamnus cathartica* cluster, the HYWIN analysis generally corroborated the discriminant analysis. Only two reference specimens, 1 and 3, were suggested as hybrids. Specimen 1, collected in England, possesses exceptionally long thorns compared with the *R. cathartica* specimens collected in North America. In a rerun of the HYWIN analysis with the exclusion of character 10 (thorn length), specimen 1 was not suggested as a hybrid. In an additional run of the data set, in which the characters having missing data were excluded, specimen 3 was not suggested as a hybrid. In the *R. utilis* cluster four reference specimens—35, 36, 38, and 39—were suggested as hybrids by the HYWIN analysis. However, the putative hybridity of these specimens should be regarded as inconclusive because of incomplete specimens.

The discriminant analysis, the HYWIN analysis of the study specimens, and the comprehensive HYWIN analysis (see Table 2) all suggested identical status for 23 of the 32 study specimens. Discrepancies between the two HYWIN analyses appeared in six specimens—41, 44, 55, 61, 63, and 72—that are all in the *Rhamnus cathartica* cluster but display some variation in blade shape (Figure 2). These specimens were not suggested as putative hybrids in the analysis using only the study specimens, but were suggested as putative hybrids in the comprehensive HYWIN analysis. Discrepancies between the comprehensive HYWIN analysis and the discriminant analysis appeared in eight specimens: 41, 44, 48, 53, 55, 61, 72 (all in the *R. cathartica* cluster), and 69

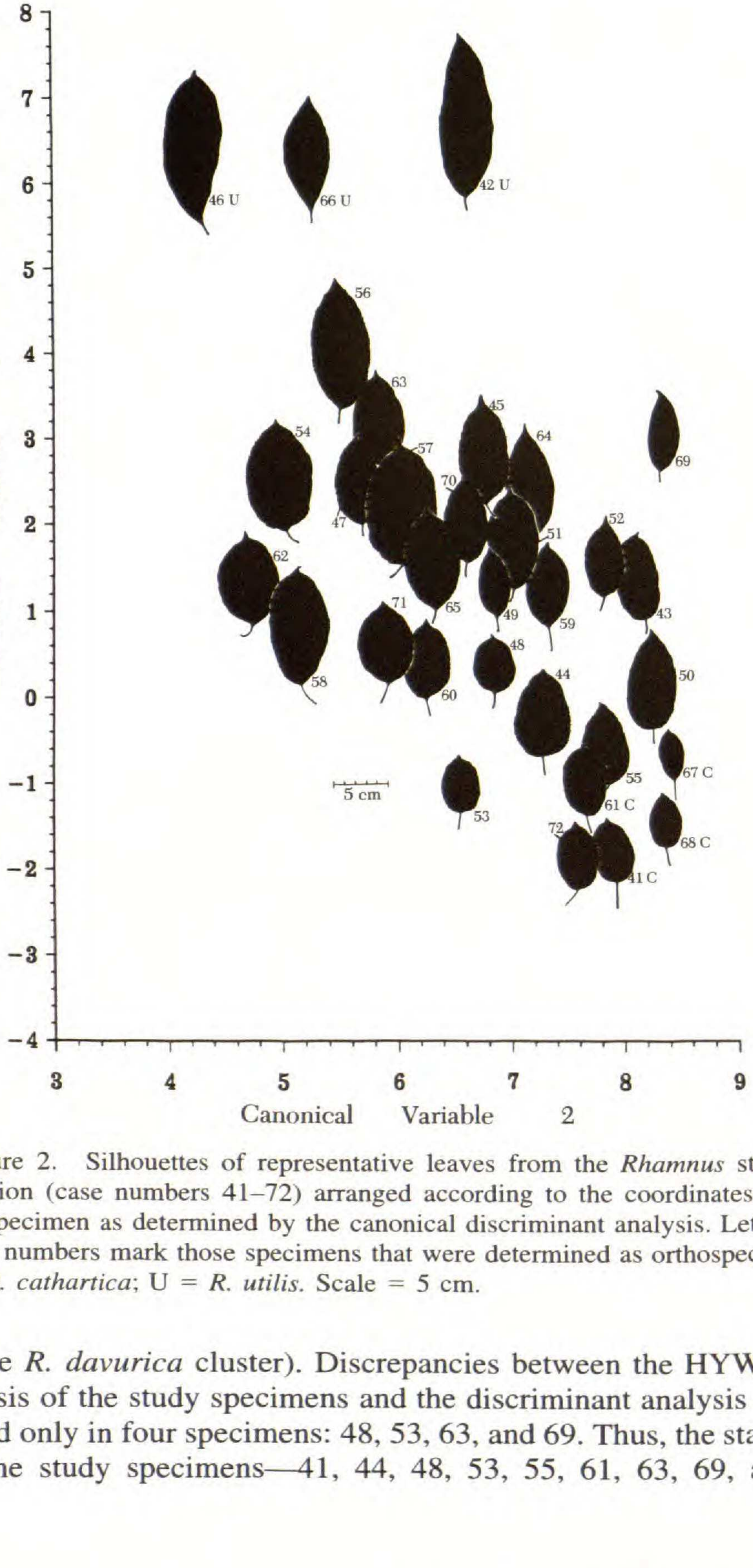

Figure 2. Silhouettes of representative leaves from the *Rhamnus* study collection (case numbers 41–72) arranged according to the coordinates for each specimen as determined by the canonical discriminant analysis. Letters beside numbers mark those specimens that were determined as orthospecies. C = *R. cathartica*; U = *R. utilis*. Scale = 5 cm.

(in the *R. davurica* cluster). Discrepancies between the HYWIN analysis of the study specimens and the discriminant analysis appeared only in four specimens: 48, 53, 63, and 69. Thus, the status of nine study specimens—41, 44, 48, 53, 55, 61, 63, 69, and

72—appeared uncertain as a result of discrepancies among the three primary analyses (see Table 2).

In the third HYWIN analysis of the study specimens, with the putative hybrids of the second analysis removed, specimen 41 was not suggested as a hybrid. The same result was obtained in an additional run of the comprehensive data set from which characters having missing data were excluded. Furthermore, in the third HYWIN analysis of the study specimens, numbers 44 and 55 were suggested as hybrids whose best matches for parental morphologies in the data set were specimens 68 and 42 [the equality scores (EQ) of both indicate that they are closer to specimen 68, and the distance to the nearest parent (NP) of each is 0.250 and 0.179, respectively]. Specimen 48 was suggested as a hybrid whose best matches for parental morphologies were specimens 66 and 68 (its equality score indicates that it is closer to specimen 68, and its distance to this nearest parent is 0.21). Specimen 48 was also suggested as a hybrid in the second run of the comprehensive data set with the putative hybrids suggested by the initial analysis removed. Specimen 61, once again, was not suggested as a hybrid. Specimen 72 was suggested as a hybrid whose best matches for parental morphologies were specimens 55 and 67 (its equality score indicates that it is closer to specimen 55 with a distance of 0.162 to this nearest parent). Specimens 53 and 69 were suggested as hybrids in all HYWIN analyses, but were included, respectively, in the *Rhamnus cathartica* and *R. davurica* var. *davurica* clusters of the discriminant analysis.

Of the 12 specimens that were never suggested as putative hybrids in the HYWIN analysis of the study specimens, only specimen 63 did not appear within the *Rhamnus utilis* or *R. cathartica* clusters of the discriminant analysis (Figure 1). Although specimen 63 was suggested 25 times as a parent, it appeared only once in this role in the highest ranking triplet of a specimen that was suggested as a hybrid (53; Table 2). Specimen 63 also was suggested as a hybrid in the comprehensive HYWIN analysis. In a subsequent run of the data set of the study specimens with the putative hybrids of the initial analysis removed, specimen 63 appeared as a putative hybrid for the first time at rank 5 and was the most frequent specimen (14 times) to be suggested as a hybrid. Its highest ranking triplet suggested that the best matches for parental morphologies in the data set were specimens 42 (*R. utilis*) and 67 (*R. cathartica*). Its equality score indicates that it

is closer to specimen 67, and its distance to this putative parent is relatively large (NP = 0.4) compared to the distance of each of the other putative hybrids to its nearest putative parent (NP = 0.1–0.3). This distance is also reflected in the discriminant analysis (Figure 1).

DISCUSSION

Age determination suggests that the site was colonized by *Rhamnus* circa 1952, and perhaps that *R. utilis* arrived at the site shortly after *R. cathartica.* Observations of numerous *Rhamnus* seedlings in the area showed that, under good conditions, fruits may occur as early as 6–8 years after seedling establishment. This would allow 5–7 generations since the introduction of the founding trees. No dead stumps or old, decrepit trees that might suggest an earlier introduction to the site were present. There is no evidence that *Rhamnus* was planted at the site. Abandonment of the orchard at the site probably instigated the proliferation of other plants, including *Rhamnus*. *Rhamnus* seeds are dispersed primarily by birds (Barnes and Wagner 1981; Brizicky 1964; Hernandez 1993; Catling and Porebski 1994). It is likely that birds, carrying the seeds from nearby *Rhamnus,* roosted on the dominant trees at the site and contributed to the initial establishment of seedlings under and at the edges of the canopy. Later, additional dispersal and establishment of seedlings resulted in the complex population present at the site. The hybrids appear very vigorous and outnumber the parents.

The results of the discriminant analysis are best interpreted as showing a variable hybrid swarm. The spatial arrangement of the clusters in Figure 1 suggests that *Rhamnus utilis* and *R. cathartica* were involved in the formation of the hybrids, and that *R. davurica* and *R. davurica* var. *nipponica* were not involved. The parental species have become connected phenetically by numerous intermediate types, presumably by backcrossing and by the production of F_2 and later hybrid generations, thereby grading one into the other. Leaf size and shape differ considerably between *R. cathartica* and *R. utilis,* but Figure 2 effectively shows that a continuum of morphology has been achieved. The placement of specimen 40—collected near the study site—between the *R. utilis* and *R. cathartica* clusters suggests that it is a hybrid.

Placement of study specimen 69 in the *Rhamnus davurica*

Table 2. Results of the first run of the HYWIN analysis of the study specimens, and a comparison of this analysis to the comprehensive HYWIN analysis and to the discriminant analysis. The highest ranking triplets of each of the 32 study specimens among the 201 highest ranking triplets at 0.95 probability. The weights assigned for the ranking criterion: wI = 1.0, wE = 1.0, wP = 1.0. Abbreviations: Rank = Rank of first time suggested as a hybrid; IN = intermediacy score; EQ = equality score; PD = parental distance score; NP = distance to the nearest parent. When the EQ value is positive, the putative hybrid is closer to putative parent 2; when it is negative, the putative hybrid is closer to putative parent 1. An underlined collection number of a putative parent denotes that the respective putative hybrid is closer to this parent; STUDY HYWIN = the HYWIN analysis of the study specimens only; COMP HYWIN = the comprehensive HYWIN analysis of 55 reference and study specimens; DA = the discriminant analysis of the reference and study specimens; O = a putative orthospecies; H = a putative hybrid; C = *Rhamnus cathartica*; U = *R. utilis*; D = *R. davurica*. The putative species identities listed in the DA column were determined according to the location of the respective specimen in relation to the species cluster in the discriminant analysis (Figure 1) as circumscribed by the dotted lines.

Case Number	Putative Parent 1	Putative Parent 2	Rank	IN	EQ	PD	NP	STUDY HYWIN	COMP HYWIN	DA
41								O	H	C
42								O	O	U
43	66	68	1	0.479	−0.003	0.677	0.358	H	H	H
44								O	H	C
45	46	68	21	0.441	−0.101	0.662	0.314	H	H	H
46								O	O	U
47	46	68	12	0.444	0.047	0.662	0.317	H	H	H
48								O	O	H
49	46	68	82	0.330	−0.214	0.662	0.313	H	H	H
50	46	68	31	0.330	0.061	0.662	0.335	H	H	H
51	61	66	2	0.571	−0.036	0.613	0.333	H	H	H
52	46	68	3	0.471	0.007	0.662	0.353	H	H	H
53	63	68	124	0.348	0.234	0.601	0.252	H	H	C
54	66	68	77	0.157	−0.043	0.677	0.374	H	H	H

Table 2. Continued.

Case Number	Putative Parent 1	Putative Parent 2	Rank	IN	EQ	PD	NP	STUDY HYWIN	COMP HYWIN	DA
55								O	H	C
56	41	46	34	0.371	0.074	0.615	0.314	H	H	H
57	66	68	71	0.185	−0.056	0.677	0.350	H	H	H
58	41	66	135	0.087	0.026	0.636	0.371	H	H	H
59	46	68	11	0.525	0.123	0.662	0.288	H	H	H
60	62	68	165	0.386	0.096	0.375	0.176	H	H	H
61								O	H	C
62	46	68	58	0.211	−0.032	0.662	0.352	H	H	H
63								O	H	H
64	46	68	4	0.492	−0.042	0.662	0.331	H	H	H
65	46	68	10	0.449	0.044	0.662	0.330	H	H	H
66								O	O	U
67								O	O	C
68								O	O	C
69	46	68	62	0.380	−0.208	0.662	0.277	H	H	D
70	66	68	7	0.463	−0.045	0.677	0.343	H	H	H
71	66	68	88	0.106	0.012	0.677	0.398	H	H	H
72								O	H	C

Table 3. The HYWIN summary output of the 201 highest ranking triplets at 0.95 probability of the data set of the study specimens. Note: "—" denotes never suggested as a hybrid or parent.

Case Number	Number of Times Ranked as a Hybrid	Rank of First Time Suggested as a Hybrid	Number of Times Ranked as a Parent	Rank of First Time Suggested as a Parent
51	25	2	0	—
64	18	4	0	—
43	17	1	0	—
52	15	3	0	—
65	14	10	0	—
47	14	12	0	—
70	12	7	0	—
57	11	71	1	166
54	10	77	0	—
56	10	34	0	—
59	10	11	1	166
45	9	21	0	—
49	9	82	0	—
50	6	31	0	—
62	6	58	1	165
69	5	62	0	—
71	5	88	0	—
58	2	135	0	—
53	2	124	2	91
60	1	165	4	112
44	0	—	9	107
42	0	—	24	27
63	0	—	25	33
48	0	—	3	60
41	0	—	40	17
66	0	—	70	1
67	0	—	5	86
68	0	—	58	1
61	0	—	34	2
46	0	—	80	3
55	0	—	17	54
72	0	—	28	8

var. *davurica* cluster (Figure 1) merits consideration. This placement and the resemblance of specimen 69 in its overall morphology to some of the reference specimens in the *R. davurica* var. *davurica* cluster suggest that it is *R. davurica* var. *davurica*. In the context of the study collection, however, this single specimen showing some characters of *R. davurica* var.

davurica is most parsimoniously explained as being a somewhat unusual hybrid. Additional specimens referable to both *R. utilis* and *R. cathartica* are represented both in the study collection and in other areas nearby, but only this single incidence of *R. davurica*-like morphology is known. In addition, there is no evidence in Figure 1 of *R. davurica* var. *davurica* acting as a parent in hybridizations. This morphology, produced in the context of a hybrid swarm, should lead to caution in identifying other collections of introduced *Rhamnus* in North America. The hypothesis that specimen 69 is a hybrid is corroborated by the results of both HYWIN analyses (Table 2). Specimen 69 was not suggested as a parent of any hybrid in either analysis. Its equality score (−0.208) indicates that it is closer to study specimen 46 of the *R. utilis* cluster. Its distance to the nearest parent (NP = 0.277) indicates that it is closer to specimen 46 than specimens 45, 49, 62, and 64 (NP = 0.314, 0.313, 0.352, and 0.331, respectively), which share the same putative parents. Specimen 53 represents a similar case in which both primary HYWIN analyses suggested that it is a putative hybrid, whereas the discriminant analysis placed it within the *R. cathartica* cluster. It appears somewhat removed from the other specimens of the *R. cathartica* cluster and deviates in a number of characters (e.g., blade length/width ratio and gloss) from the specimens circumscribed as orthospecies. The results of the HYWIN analyses strongly support the hypothesis that it is a hybrid.

The third HYWIN analysis of the study specimens with the hybrids suggested by the second analysis removed, the discriminant analysis, and the comprehensive HYWIN analysis all support the hypothesis that specimen 63 is a hybrid of *Rhamnus utilis* and *R. cathartica.*

On the basis of the third HYWIN analysis of the study specimens, specimens 41 and 61 are hypothesized as *Rhamnus cathartica,* whereas specimens 44, 48, 55, and 72 are hypothesized as hybrids. The low NP values of specimens 44 and 55 may indicate that they resulted from backcrossing to *R. cathartica.* The suggestion that specimen 72 resulted from hybridization of plants resembling specimens 55 and 67 reinforces the interpretation of the presence of a variable hybrid swarm at the study site. In addition, this suggestion sheds light on the difficulties in determining whether specimen 72 is an ortho-

species or a hybrid since a putative backcross (specimen 55) has most likely hybridized with *R. cathartica* and produced a plant whose morphology is very close to that of *R. cathartica.*

One additional aspect of Figure 1 merits brief mention. The spatial separation of *Rhamnus davurica* var. *davurica* and *R. davurica* var. *nipponica* (Figure 1) is relatively clear, perhaps as clear as that of *R. davurica* var. *davurica* and *R. utilis.* Though very preliminary and based on a small sample size, this result supports the recognition of *R. davurica* var. *nipponica* as a species, *R. nipponica* (Makino) Grubov (1949a). Schneider (1916) postulated that *R. davurica* is most nearly related to *R. cathartica,* and pointed out the difficulty of determining whether *R. davurica* var. *nipponica* is a good variety or even a different species. Choo et al. (1993) discovered significant differences in the pollen grains of *R. davurica* var. *davurica* and *R. davurica* var. *nipponica* and suggested that their taxonomic rank should be amended pending additional, broader investigation. Drawing taxonomic conclusions from these preliminary observations is premature, but we hope that they may point the way to additional research.

The discriminant analysis provides an effective visual presentation of the parental species and putative hybrids. The HYWIN analyses provide "fine tuning" in cases where the results of the discriminant analysis are not clear enough to allow confident placement of specimens at the boundaries of clusters of species. Several study specimens were placed within the orthospecies cluster by the discriminant analysis, but were suggested as hybrids by the more stringent HYWIN analyses. These discrepancies are suggestive of backcrosses closely resembling parents, and here are so interpreted. Thus, comparison of the results of both types of analyses enables the formulation of more precise hypotheses. The utilization of both analyses in studies of hybrid swarms appears to be very effective.

The results of all three analyses allow us to conclude that three study specimens—42, 46, and 66—can be identified as *Rhamnus utilis*; four study specimens—41, 61, 67, and 68—as *R. cathartica,* and 25 specimens—43–45, 47–60, 62–65, and 69–72—can be designated putative hybrids of these two species (Figure 2).

Reconstruction of the actual complex hybridization events that resulted in the full range of variation present at the study site's

hybrid swarm is not possible. However, the analyses presented enabled the identification of the orthospecies and their results may serve as the best hypotheses to account for the most recent hybridization event that produced the morphology of each of the putative hybrids.

It is interesting to note that two Old World species that have been brought together to a habitat on a different continent, away from their place of origin, have hybridized naturally and probably produced several generations of fertile hybrids. The first step of this evolutionary progression involved human intervention, but the next steps have occurred without that intervention. The aggressive nature of these new hybrids should be of concern to local stewards and naturalists.

Wagner (1983) pointed out that if the number of taxa involved in a study is large and the characters that separate them are poorly differentiated, the problems of detection of hybrids may be severe. We hope that the methodology and results of this study encourage researchers to conduct morphological analyses of complex hybrid swarms in other taxa.

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APPENDIX 1: SPECIMENS EXAMINED

NOTE: The case numbers (in parentheses below) are used in the text and in Figure 1 to abbreviate the collectors and collection numbers.

STUDY SPECIMENS

U.S.A. MICHIGAN. Washtenaw County: Ann Arbor, Leslie Science Center Park, 6 Sep. 1984, *Reznicek & Reznicek 7462* (41); *7463* (42); *7464* (43); *7465*

(44); *7466* (45); *7467* (46); *7468* (47); *7469* (48); *7470* (49); *7471* (50); *7472* (51); *7473* (52); *7474* (53); *7475* (54); *7476* (55); *7477* (56); *7478* (57); *7479* (58); *7480* (59); *7481* (60); *7482* (61); *7483* (62); *7484* (63); *7485* (64); *7486* (65); *7487* (66); *7488* (67); *7489* (68); *7490* (69); *7491* (70); *7492* (71); *7493* (72) (MICH).

REFERENCE SPECIMENS

***RHAMNUS CATHARTICA* L.**

ENGLAND. Nottingham County: near Newark, Sep. 1890, *Fisher s.n.* (1) (MICH). **U.S.A.** ILLINOIS. Cook County: Burre Woods, 26 Aug. 1960, *Laskowski 187* (6) (MICH). MICHIGAN. Emmet County: on the west bank of Tannery Creek, T35N, R5W, Sec. 28, Oct. 1967, *Tanton s.n.* (4) (MICH). Hillsdale County: west side of Bankers Road, T7S, R3W, Sec. 6, 14 Sep. 1985, *Fritsch 115* (5) (MICH). Oakland County: Broomfield Hills, 3 Sep. 1916, *Billington s.n.* (2) (MICH); waste places and along roads near Birmingham, 29 Aug. 1915, *Chandler s.n.* (3) (MICH). Washtenaw County: Ann Arbor, along the banks of Huron River, 14 Oct. 1949, *Jordal 3339* (7) (MICH). NEW YORK. Cayuga County: Salmon Creek, south of Genoa, 28 Aug. 1919, *Eames & Wiegand 12422* (9) (MICH). Orange County: Black Rock Forest, along Old Point Road near Upper Res., 22 Jul. 1966, *Raup 7668* (8) (MICH). OHIO. Clark County: Springfield Township, Sec. 4, NE ¼, North of Beaver Creek, 0.15 mile S of Tuttle Cemetery and 0.3 mile NW of Redmond Road Bridge, 23 Aug. 1983, *Cusick 22927* (10) (MICH).

RHAMNUS DAVURICA* PALL. VAR. *DAVURICA

CHINA. Jilin Province: lake shore, 27/31 Jul. 1931, *Chen 170* (20) (A). Shanxi Province: Chih-li, 12 Oct. 1924, *Dorsett 967* (21) (A). Zhejiang Province: Hsiao Lin, 30 Aug. 1925, *Dorsett 4110* (24) (A). Manchuria: Halasust, along Yalu River, 10 Aug. 1929, *Skvortzov s.n.* (18) (A). **KOREA.** Kyonggi-Do, Kwangnung, 37°44′N, 128°06′E, 15 Oct. 1947, *Chung 788* (12) (MICH). Kankyo Province: Sempo, 3 Aug. 1917, *Wilson 8827* (25) (A). **RUSSIA.** Manchuria: Khabarovsk area, mouth of the River Kur at the Amur River, 13 Sep. 1895, *Komarov 1072* (22) (A).

***RHAMNUS DAVURICA* PALL. VAR. *NIPPONICA* MAKINO**

CHINA. Hubei Province: Lichuan Xian, Hsien, vicinity of Zhuanjiaowan, 30°10′N, 108°45′E, *Sino-American Botanical Expedition 2024* (23) (A). **JAPAN.** Honshu: near Yamanaka Lake in Kai, 18 Jul. 1956, *Togasi 1365* (14) (MICH). Pref. Nagano. Shinano Province: Mikuni-tooge and Mikuni-tooge Road, Azusayama Kawakami-mura, Minami-saku-gun, 4 Sep. 1960, *Furuse s.n.* (29) (A); Mount Temgu Kawakami-mura, Minami-saku-gun 19 Sep. 1962, *Furuse s.n.* (26) (A); Minami-karuizawa, 18 Aug. 1951, *Mizushima 10209* (30) (A); Minamisaku-gun, Nobeyama, 31 Jul. 1971, *Togashi & Tateishi 73* (27) (A); Nobeyamagahara, East foot of Mount Yatsuga-dake, Minamisaku-gun, 31 Jul. 1972, *Tateishi & Togashi 239* (28) (A). **KOREA.** Kwangnung: Kyonggi-Do, 28 Sep. 1933, *Chung 5588* (11) (MICH). Cholla-Namdo: Mudung San, 35°5′N, 127°E, 2 Oct. 1981, *Meyer & Bristol 164* (19) (A).

***RHAMNUS UTILIS* DECNE.**

CHINA. Anhui Province: Siunin, 7 Sep. 1925, *Ching 3318* (35) (A). Fujian Province: Kuliang, 27 Jul. 1926, *Chung 6704* (36) (A). Guangdong Province: Yu-yuen, 20 May 1933, *Ko 52678* (38) (A); Loh Ch'ang District, Chong Uen Shan near Kau Fung, 2–30 Nov. 1932, *Tsang 20642* (17) (MICH). Guangxi Province: Kwei-lin District, Hsi-chang village and vicinity, Ch'i-fen-shan, 1–11 Oct. 1937, *Tsang 28408* (37) (A). Guizhou Province: Jiangkou Xian, along the Yixi River between Guanba and Gaofeng, SW side of the Fanjing Shan mountain range, 5 Sep. 1986, *Sino-American Guizhou Botanical Expedition 912* (16) (MICH); Kiangkou Hsien, Miao Wang, 26 Sep. 1931, *Steward et al. 532* (32) (A); Songtao Xian, NE of Fanjing Shan mountain range, 5–6 Oct. 1986, *Sino-American Expedition 1858* (15) (MICH). Hubei Province: Hsing-shan, Hsien, Nov. 1907, *Wilson 623* (33) (A); Shennongjia Forest District, 31°30′N, 11°30′E, Laojunshan Yaowan Canyon on the W side of the Jiuchong River, ca. 1 km. S of Mucheng, 31 Aug. 1980, *Sino-American Expedition 465* (31) (A). Zhejiang Province: S of Pang Yung, 10 Jul. 1924, *Ching 2054* (34) (A). **U.S.A.** MICHIGAN. Washtenaw County: Ann Arbor, end of Wickfield Court between Traver Road and Pontiac Trail, 25 Aug. 1980, *Reznicek 6165* (39) (MICH).

ADDITIONAL SPECIMEN EXAMINED

U.S.A. MICHIGAN. Washtenaw County: Ann Arbor, Nichols Arboretum, N of Geddes Avenue, 6 Sep. 1984, *Reznicek 7461* (40) (MICH).